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Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. III. Influence on Gas Exchange in the Guinea Pig Lung

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The influence of methyl isocyanate (MIC) inhalation on the gas exchange function of the lungs in guinea pigs was studied by measuring arterial blood gases, pH, and tracheal pressure during constant-volume, artificial ventilation with air or 100% O_2 at 40 and 120 min after exposure. A 15 min exposure to MIC at concentrations of 240 to 628 ppm caused a marked reduction in Pa_{O_2} and pH_a and an elevated tracheal pressure during artificial ventilation. The low Pa_{O_2} was only slightly elevated when the animals were ventilated with 100% O_2. Although the dry-wet lung weight ratio was reduced at the highest exposure concentration, the effect was not severe and no significant increase in lung water was found at the lower concentrations. MIC inhalation caused severe pulmonary blood shunting and ventilation/perfusion imbalance. This, in turn, led to hypoxemia, metabolic acidosis, and tissue hypoxia, which could produce death. The pulmonary gas exchange deficit likely resulted from bronchial and bronchiolar obstruction caused by sloughed epithelium and other debris from intra- and extrapulmonary airways.

Introduction

Following inhalation exposure to methyl isocyanate (MIC) at levels of 30 to 200 ppm, rats quickly exhibit respiratory distress with a slow, gasping breathing pattern (*1,2*). There is nasal and eye irritation, fluid discharge from the nose, and extensive damage to the epithelium in both upper and lower airways. These animals also exhibit a reduction in arterial pH (pH_a) and partial pressure of oxygen (Pa_{O_2}), and a rise in arterial partial pressure of carbon dioxide (Pa_{O_2}), which is especially pronounced in animals that die during exposure.

The changes in arterial blood gases and pH may result because of damage to the respiratory control system, leading to hypoventilation, and/or because of gas exchange impairment in the lung. The current study was undertaken to determine if blood gases and pH were altered following inhalation of MIC, when the ventilation of the lung was held constant by use of a constant-volume respirator. We further attempted to identify the cause of rapid death in guinea pigs following exposure to high concentrations.

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Methods

Animals and Animal Preparation

Seven adult, specific pathogen-free, Hartley, female guinea pigs (Hazleton Research Animals, Denver, PA), with an average body weight of 442 ± 51 g, were exposed to concentrations of MIC ranging from 240 to 628 ppm for 15 min in a static exposure chamber (*3*). Four control guinea pigs were studied without exposure to MIC. Immediately after removal from the exposure chamber, the animals were placed in a small enclosure containing 5% halothane in air. When the animals were in a surgical plane of anesthesia, their tracheae were cannulated and the animals were artificially ventilated with a constant-volume pump (Harvard Instruments, model 683) at a tidal volume of 3mL and a frequency of 76 breaths/min. These values were chosen to slightly hyperventilate the lungs of the control animals, compared to previous studies on unanesthetized, spontaneously breathing guinea pigs (*4*), with the goal of maintaining an optimal level of gas exchange in the anesthetized animals. A light surgical plane of anesthesia was maintained by flowing air containing 1.5% halothane (Fluotec Mark III vaporizer) past the inlet of the respirator at a flow slightly higher than the peak inspiratory flow (Fig. 1). The gas was humidified at room temperature by bubbling through water.

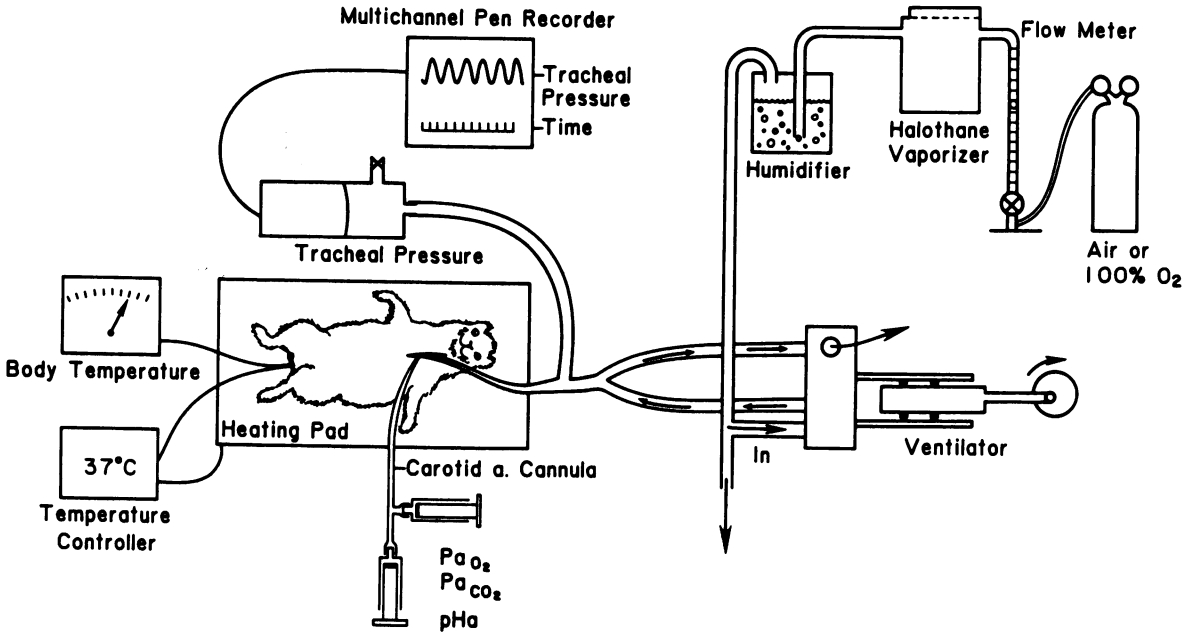

FIGURE 1. Experimental arrangement for constant-volume artificial ventilation of anesthetized guinea pigs following MIC inhalation.

The left common carotid artery was cannulated (PE 50) for withdrawal of blood for blood gas and pH measurement. Body temperature was measured with a calibrated rectal thermistor inserted 6 cm into the lower gastrointestinal tract and a telethermometer (Yellow Springs Instrument, model 43). The signal from a second thermistor placed at the same depth was used by a temperature controller (Yellow Springs Instrument, model 72) to operate a heating pad to hold the body temperature near 37°C.

Measurements

Arterial blood gases and pH were measured using a Corning Model 170 blood gas analyzer that was calibrated with analyzed gases supplied by the manufacturer. Measurements were made at 37°C but were corrected to the animal's body temperature when differences occurred. The pressure induced by inserting 3 mL of gas into the lungs was measured at the tracheal cannula with a pressure transducer (Gould, model P23 ID). The changes in pressure were recorded on a multichannel pen recorder (Grass, model 7D).

Blood samples were also taken for measurement of total white cells, erythrocytes, hematocrit, and hemoglobin on a Coulter Counter S-Plus IV. At the end of the experiment, the animals were killed with an overdose of sodium pentobarbital and the lungs were removed, examined for gross lesions, weighed, and dried for 72 to 96 hr at 50°C until further drying resulted in no additional reduction in weight. The dry/wet lung weight ratio was calculated for each animal.

Periodic Hyperinflation of the Lungs (Sighing)

In preliminary experiments on two control animals, it became apparent that gas exchange deteriorated after 2 hr of artificial ventilation (Table 1). There was a substantial reduction in Pa_{O_2} by this time. Subsequent animals were periodically hyperinflated by occluding the outflow of the pump for 3 to 4 tidal volumes twice at 20-min intervals and about 5 min prior to making measurements. This procedure, referred to as sighing, resulted in marked improvement of the Pa_{O_2} and resulted in no measurable deterioration of gas exchange function with time. Further, no areas of atelectasis were observed in the lungs of the control animals that were sighed, although such areas were observed in those that were not sighed. It appears that sighing is extremely important in maintaining normal gas exchange function in artificially ventilated guinea pigs.

Protocol

The animals were weighed and either exposed to MIC or directly anesthetized and studied. Following exposure to MIC, the guinea pigs were anesthetized and artificially ventilated with air and halothane. The first measurement of blood gases and other variables was made about 40 min after exposure and was repeated at 120 min after exposure. The air was then replaced by 100% oxygen, and the animals were ventilated for an additional 20 min. All measurements were then repeated. The animals were then killed and a necropsy was performed.

Table 1. Changes in blood gases during artificial ventilation with and without sighing; 0 ppm, air, means ± SD.

Treatment	n[a]	Ventilation time, min	Pa_{O_2}, torr	Pa_{CO_2}, torr	pH_a	$[HCO_3^-]$, mmole/L
Without sighing	2	40	74.7 ± 4.7	33.9 ± 0.4	7.38 ± 0.06	20.3 ± 2.4
With sighing	4	40	78.2 ± 4.2	31.3 ± 2.4	7.42 ± 0.04	20.0 ± 2.0
Without sighing	2	120	61.6 ± 0.8	36.4 ± 7.1	7.40 ± 0.06	22.6 ± 1.5
With sighing	4	120	89.3 ± 4.9*	28.5 ± 0.8	7.44 ± 0.01	19.5 ± 1.1*

[a] n = number of animals studied.
* Significantly different from animals not sighed, $p < 0.05$.

Data Analysis

A one-way analysis of variance was applied to data for each variable from the various treatment groups at each of the three measurement periods to determine if the various MIC treatments resulted in a significant change ($p < 0.05$). If a significant F value was obtained, a least significance difference test was used to determine which treatments differed from each other. All results are reported as means ± standard deviation (SD).

Results

Rapid, detrimental effects on gas exchange and pH were found at all MIC exposure concentrations used (Table 2). Pa_{O_2} and pH_a were severely decreased in all cases, even though a constant volume of gas entered the trachea. Although Pa_{O_2} increased with the increase in exposure level when the first measurements were made, it returned to nearly normal levels by 2 hr after exposure. Because the acidosis remained and the bicarbonate concentration dropped at the latter time, it is clear that a major component of the acidosis resulted from production of fixed acids and was, therefore, of metabolic origin. Pa_{O_2} and pH_a did not appear to become worse with time after exposure and no animals died while being artificially ventilated.

To determine if the low Pa_{O_2} after MIC exposure was the result of intrapulmonary blood shunting or ventilation/perfusion inequality in the lung, we ventilated the animals for 20 min using 100% O_2 and repeated the arterial blood gas and pH measurements. The expected high Pa_{O_2} was observed in guinea pigs not exposed to MIC, but dramatically reduced values occurred in all treated animals (Table 3). The low Pa_{O_2} values suggest the likelihood of severe intrapulmonary blood shunting following MIC exposure but, because the Pa_{O_2} was somewhat improved over that during air-breathing, there was also some degree of ventilation/perfusion inequality.

Marked increases in tracheal pressure during constant volume ventilation following MIC exposure indicates likely reduction in the compliance of the lung (Fig. 2 and Table 4). Direct observation of the chest of these animals indicated less expansion in the exposed animals than in the controls.

Although a significant decrease in the lung dry/wet ratio (13% change) was found at the highest MIC exposure, the increase in water content was not dramatic (Table 5). There was no change in lung water content in the 240 ppm exposure group and only an 8.5% change in the 526 ppm group. These data suggest that even though the animals were exposed to high concentrations of MIC, the immediate disturbance in gas exchange was not caused by excess fluid in the lung.

During the course of the experiment, a small amount of blood was lost during surgery and approximately 2 mL of blood were taken for analyses. In addition, heparinized saline was injected to keep the catheter from clotting. To be certain that this procedure did not upset the hemodynamic balance of the animals, we measured several variables associated with O_2 transport, namely hematocrit (Hct), hemoglobin (Hb), and red blood cell (RBC) count. Neither the experimental procedure nor

Table 2. Effects of acute MIC exposure on arterial blood gases during artifical ventilation with air, means ± SD.

MIC exposure, ppm	n[a]	Time post-exposure, min	Pa_{O_2}, torr	Pa_{CO_2}, torr	pH_a	$[HCO_3^-]$, mmole/L
0	4	40	78.2 ± 4.2	31.3 ± 2.4	7.42 ± 0.04	20.0 ± 2.0
240	2	40	41.8 ± 9.0*	35.8 ± 13.2	7.26 ± 0.08*	15.5 ± 3.0
526	2	40	33.1 ± 7.2*	47.1 ± 9.2*	7.25 ± 0.01[a]	20.8 ± 4.2
628	3	40	32.4 ± 3.2*	54.1 ± 1.7*†	7.09 ± 0.05*†‡	16.5 ± 2.3
0	4	120	89.3 ± 4.9	28.5 ± 0.8	7.44 ± 0.01	19.5 ± 1.1
240	2	120	41.5 ± 12.0*	32.9 ± 9.8	7.17 ± 0.04*	12.2 ± 4.6*
526	2	120	35.4 ± 4.8*	40.0 ± 2.3*	7.26 ± 0.01*†	17.8 ± 0.5†
628	3	120	37.6 ± 2.8*	37.2 ± 2.1*	7.18 ± 0.05*‡	14.0 ± 1.0*

[a] n = number of animals studied.
* Significantly different from 0 ppm, $p < 0.05$.
† Significantly different from 240 ppm, $p < 0.05$.
‡ Significantly different from 526 ppm, $p < 0.05$.

Table 3. Effects of acute MIC exposure on arterial blood gases during artificial ventilation with 100% O_2, means ± SD.

MIC exposure, ppm	n[a]	Pa_{O_2}, torr	Pa_{CO_2}, torr	pH_a	$[HCO_3^-]$, mmole/L
0	4	491 ± 36	26.4 ± 0.6	7.46 ± 0.02	19.0 ± 1.2
240	2	80.1 ± 6.1*	35.0 ± 1.6	7.12 ± 0.10*	11.7 ± 3.2*
526	2	60.1 ± 5.1*	35.2 ± 1.6	7.27 ± 0.03*†	16.3 ± 1.8†
628	3	63.9 ± 19.4*	40.9 ± 11.0*	7.15 ± 0.08*	14.0 ± 1.4*

[a] n = number of animals studied.
* Significantly different from 0 ppm, $p < 0.05$.
† Significantly different from 240 ppm, $p < 0.05$.

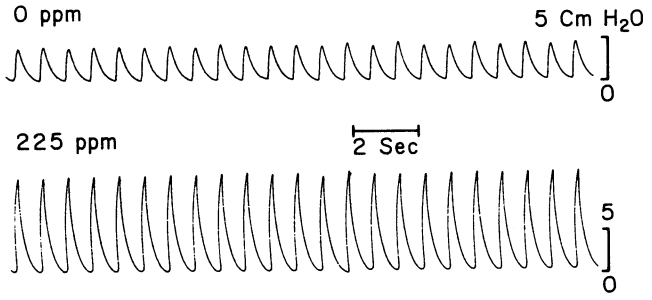

FIGURE 2. Recordings of tracheal pressure during constant-volume artificial ventilation of a control guinea pig (top tracing) and a guinea pig exposed to 225 ppm MIC (bottom tracing). Upward deflection occurs during inflation of the lungs.

Table 4. Changes in tracheal pressure during constant volume artificial ventilation with acute MIC exposure; 120 min post-exposure, means ± SD.[a]

MIC exposure, ppm	n[a]	Tracheal pressure, cm H_2O
0	4	4.3 ± 0.5
240	2	16.3 ± 1.0*
526	2	13.4 ± 1.6*
628	3	17.7 ± 3.4*‡

[a] n = number of animals studied.
* Significantly different from 0 ppm, $p < 0.05$.
‡ Significantly different from 526 ppm, $p < 0.05$.

Table 5. Changes in lung water content after acute MIC exposure, means ± SD.

MIC exposure, ppm	n[a]	Dry/wet weight ratio
0	4	0.200 ± 0.005
240	2	0.201 ± 0.011
526	2	0.183 ± 0.006
628	3	0.174 ± 0.014*†

[a] n = number of animals studied.
* Significantly different from 0 ppm, $p < 0.05$.
† Significantly different from 240 ppm, $p < 0.05$.

the MIC treatment caused an alteration in number of RBCs, Hb, or Hct. Previous studies on spontaneously breathing guinea pigs exposed to MIC suggest the occurrence of hemoconcentration (*5*). It is possible the added fluids in our experiment eliminated that effect. There was a significant elevation in white blood cell count in the highest MIC-treated group, but the cause is not known.

Gross observation of the lungs of the MIC-treated guinea pigs indicated atelectasis in areas of both right and left lungs. These areas were scattered throughout the lungs and did not seem to appear in any consistent regions in all animals. There were often multifocal hemorrhages in both lungs. Upon opening the trachea and primary bronchi, long strands of viscous material could be pulled out, often in a single piece with bifurcations extending down each primary bronchus. It is likely that this material occupied a large cross-sectional area of the lumen, especially of the smaller bronchi and bronchioles. The nasal passageways were also filled with a viscous, straw-colored material. None of the untreated animals had gross signs of lung abnormalities.

Discussion

These experiments demonstrated the severe and rapid influence of MIC inhalation on the gas exchange function of the lung. Inhaling this highly reactive compound resulted in extreme hypoxemia that undoubtedly led to severe tissue hypoxia and metabolic acidosis. The latter disturbances are the probable cause of rapid death during or following high level exposure.

All evidence from this experiment suggests that the primary cause of hypoxemia following MIC inhalation is intrapulmonary blood shunting and ventilation/perfusion ($\dot{V}/\dot{Q}$) inequality. Measurement of Pa_{O_2} during inhalation of 100% O_2 provides a test for lung units that receive no ventilation but are still perfused (intrapulmonary blood shunting). When 100% O_2 is inhaled for several minutes, blood perfusing ventilated lung units equilibrates with the high alveolar P_{O_2} but has an oxygen content only slightly higher than blood equilibrated by lung units previously ventilated with air. The blood O_2 dissociation curve is nearly flat at a P_{O_2} above 100 torr. When mixed venous blood passes through lung units that are not ventilated (shunted blood), no gas exchange occurs, and the P_{O_2} and O_2 content remain low. Mixing of the latter blood with that equilibrated with high alveolar P_{O_2} results in a lowering of the O_2 content of the mixture in proportion to the amount of shunted blood. Because of the nearly flat O_2 dissociation curve at high P_{O_2}, a small reduction in O_2 content leads to a large reduction in P_{O_2} of the mixture that is easily detectable. The magnitude of the reduction in P_{O_2} can

be used to estimate the degree of the shunt. On the other hand, lung units that are poorly ventilated, relative to their perfusion ($\dot{V}/\dot{Q}$ inequality), will also contain a high alveolar P_{O_2} during the breathing of 100% O_2, as in the case of lung units with a normal ventilation, provided enough time has been given for the washout of N_2 present when air was breathed. Thus, mixed venous blood passing through these lung units will be equilibrated with a high alveolar P_{O_2} and not cause a reduction in the P_{aO_2} below that expected in a normal lung.

Because the P_{aO_2} was not elevated substantially during 100% O_2 breathing after exposure to MIC, some areas of the lung appeared to receive no ventilation but still had abundant perfusion; thus, intrapulmonary blood shunting was present. However, the P_{aO_2} of the MIC-exposed animals did rise approximately 30 torr when they breathed 100% O_2, suggesting that some lung areas were underventilated (but still receiving some ventilation) with respect to their perfusion.

The observed functional disturbances in gas exchange are consistent with bronchial or bronchiolar obstruction resulting from sloughed epithelial cells, secretions, and other debris, possibly derived from the airways proximal to the obstruction. Histopathological examination of the respiratory system following MIC exposure has demonstrated these plugs in both large and small airways (*1,6–8*). Such an obstruction is also indicated by the high tracheal pressures that accompanied the 3-mL tidal volume delivered by the respirator and has been described in rats exposed to lower concentrations of MIC (*9*).

Arterial P_{CO_2} was not excessively increased after MIC exposure in these pump-ventilated animals. This may result despite shunting and $\dot{V}/\dot{Q}$ inequality because certain areas of the lung are likely to be overventilated by the constant tidal volume, thereby, removing excess amounts of CO_2 from the blood perfusing these areas. The mixture of blood from the overventilated areas with that from underventilated (or completely unventilated areas), therefore, might be relatively normal. The lack of evidence for extensive pulmonary edema suggests that the cause of shunting and $\dot{V}/\dot{Q}$ imbalance was not alveolar flooding.

It is probable that the hypoxemia developed rapidly during MIC exposure, as indicated by the gasping breathing pattern of the animals in the chamber. That would quickly lead to tissue hypoxia and the resulting elaboration of lactic acid into the blood. The fixed acid would cause metabolic acidosis, resulting in a marked reduction in O_2 affinity of hemoglobin and a further reduction in O_2 transport to the tissues (*10*). If unchecked, this positive feedback would quickly lead to death, a common finding at exposure levels used in this study.

None of the artificially ventilated animals died during the course of this study. The tissue hypoxia and acidosis were still compatible with life, even though they were incurred in a very short time. The situation is likely to be compounded in spontaneously breathing animals because of possible impairment of the respiratory control system (*1*). Those animals very likely cannot maintain an adequate tidal volume because of the decreased compliance of the lung, irrespective of exaggerated ventilatory efforts. They then would be subject to hypoxemia and tissue hypoxia to an even greater extent than the artificially ventilated animals. Thus, prolonged survival may be possible after MIC exposure if assisted ventilation, oxygen therapy, and treatment of the metabolic acidosis are applied at an early enough time.

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